Article

Pilot study to evaluate 3 hygiene protocols on the reduction of bacterial load on the hands of veterinary staff performing routine equine physical examinations

Josie L. Traub-Dargatz, J. Scott Weese, Joyce D. Rousseau, Magdalena Dunowska, Paul S. Morley, David A. Dargatz

Abstract — Reduction factors (RFs) for bacterial counts on examiners' hands were compared when performing a standardized equine physical examination, followed by the use of one of 3 hand-hygiene protocols (washing with soap, ethanol gel application, and chlorohexidine-ethanol application). The mean RFs were 1.29 $\log_{10}$ and 1.44 $\log_{10}$ at 2 study sites for the alcohol-gel (62% ethyl alcohol active ingredient) protocols and 1.47 $\log_{10}$ and 1.94 $\log_{10}$ at 2 study sites for the chlorhexidine-alcohol (61% ethyl alcohol plus 1% chlorhexidine active ingredients) protocols, respectively. The RFs were significantly different ($P < 0.0001$) between the hand-washing group and the other 2 treatment groups (the alcohol-gel and the chlorhexidine-alcohol lotion). The use of alcohol-based gels or chlorhexidine-alcohol hand hygiene protocols must still be proven effective in equine practice settings, but in this study, these protocols were equivalent or superior to hand washing for reduction in bacterial load on the hands of people after they perform routine physical examinations.

Résumé — Étude pilote pour l'évaluation de 3 protocoles d'hygiène visant à réduire la charge bactérienne sur les mains du personnel vétérinaire effectuant des examens physiques de routine chez les chevaux. Le nombre de bactéries sur les mains d'examinateurs a été mesuré lors d'un examen physique standardisé chez des chevaux et comparé à celui obtenu en appliquant l'un des 3 protocoles d'hygiène des mains (lavage au savon, application d'un gel d'éthanol et application de chlorhexidine-éthanol) afin de mesurer les facteurs de réduction (FR) du nombre de bactéries. Les FR moyens étaient de 1,29 $\log_{10}$ et 1,44 $\log_{10}$ sur les 2 sites choisis pour les protocoles au gel d'alcool (62 % d'alcool éthylique comme ingrédient actif) et 1,47 $\log_{10}$ et 1,94 $\log_{10}$ sur les 2 sites pour les protocoles au chlorhexidine-alcool (61 % d'alcool éthylique plus 1 % de chlorhexidine comme ingrédients actifs). Les FR étaient significativement différents ($P < 0{,}0001$) entre le groupe de lavage des mains et les 2 autres groupes (gel d'alcool et lotion de chlorhexidine-alcool). L'efficacité des gels à base d'alcool ou de chlorhexidine-alcool dans les protocoles d'hygiène des mains en pratique équine n'est pas clairement établi, mais dans ce cas-ci, ces protocoles étaient équivalents ou supérieurs au lavage des mains pour la réduction de la charge bactérienne après des examens physiques de routine.

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Introduction

Proper hand hygiene has been proposed as the main means of preventing nosocomial spread of pathogens in the human hospital setting and community (1–6). Semmelweiss (7) appears to have been the first to demonstrate that improved cleansing of heavily contaminated hands with an antiseptic agent by health-care workers may reduce transmission of infectious agents.

Despite the recognized benefits of hand hygiene, it is still underappreciated and underutilized in health-care

Department of Clinical Sciences, Colorado State University, College of Veterinary Medicine and Biomedical Sciences, 300 W. Drake Avenue, Fort Collins, Colorado 80523, USA (Traub-Dargatz, Dunowska, Morley); Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1 (Weese, Roussau); Centers for Epidemiology and Animal Health, USDA: APHIS:VS, 2150 Centre Avenue, Building B, Fort Collins, Colorado 80526 USA (Dargatz).

Address all correspondence and reprint requests to Dr. Josie L. Traub-Dargatz; e-mail: jtraub1@lamar.colostate.edu

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settings, and lack of compliance with hand-hygiene protocols is a major challenge for programs focused on the control of infections. Lack of time to perform the procedure, lack of readily available means to perform the hand washing procedure, and irritation (drying of skin, cracking of skin, painful skin) of hands from hand washing are reasons often cited by health care workers for not complying with hand washing protocols between each patient contact (8).

The types of bacteria on hands can be divided into 2 categories: the transient flora and the resident flora (9). The transient flora can colonize the superficial layers of the skin and are more amenable to removal by routine hand washing. Transient bacteria are acquired by health-care workers during direct contact with patients or contact with contaminated environmental surfaces. These transient bacteria are the ones most often related to health-care-associated infections. The resident flora are associated with the deeper layers of the skin and are more difficult to remove. The resident microorganisms generally are less likely to be pathogenic to patients (10). However, even these resident flora have the potential to cause disease in immune-compromised patients. Studies of the flora on the hands of health care workers in equine practice have not been conducted, to the authors' knowledge.

Evaluation protocols for regulatory approval and advertising claims of hand-hygiene products have been conducted in vivo and in vitro, and they are often reported as the change in bacterial load as the $\log_{10}$ Reduction Fraction (RF). If there is a large bacterial load on the hands, there is more potential for an effective hygiene procedure to reduce the bacterial load and result in a larger RF than if hands have only a low number of bacteria on surfaces prior to applying the hand-hygiene procedure (11).

According to the manufacturer's advertisement, the in vitro time to killing for selected bacteria by a chlorhexidine-alcohol hand sanitizer (Avagard; 3M Health Care, St. Paul, Minnesota, USA) is 15 s for 99% of the bacteria of various types. However, it is further indicated that "the clinical significance of in vitro microbiology is unknown."

In 1995 and 1996, the Healthcare Infection Control Practices Advisory Committee recommended that either antimicrobial soap or a waterless antiseptic agent be used for sanitizing hands when leaving the rooms of human patients infected with multidrug-resistant pathogens and methicillin resistant *Staphylococcus aureus* (MRSA) (8). The Centers for Disease Control and Prevention's guidelines for hand hygiene in the health-care setting mention that alcohol-based products are more effective than hand washing with antibacterial soap (8). Alcohol-based hand rubs for the postcontamination treatment of hands of health-care workers have many advantages over antimicrobial soaps, including spectrum of antimicrobial activity; speed of antimicrobial activity and efficacy; dermal tolerance; convenience of use; and, finally, the favorable impact on reduction of prevalence of hospital-acquired infections, particularly those caused by MRSA (11,12). Liquid and gel hand rubs are also recommended for use in human hospitals in Europe (13–16). If compliance is better with an alcohol-based hand gel than with hand washing with an antibacterial soap, it is possible that, in the clinical setting, hand gel may have a more favorable impact on infection control (12).

Veterinary patients likely have a higher bacterial load on their body surfaces than do most human patients. Reasons for this include the following: 1) they are not bathed regularly; 2) they are haired over most of their bodies; and 3) they often reside in close proximity to their body wastes (feces and urine), at least for large animals. To our knowledge, the typical bacterial load on the skin surfaces of domestic animals has not been quantified, and the typical change in bacterial load on hands of health-care personnel associated with performing a common procedure, such as a physical examination on a horse, has not been reported.

The hands of animal caretakers and health care workers have been proposed as a means of spreading bacterial and viral agents that are pathogenic to hospitalized patients. For equine patients, examples include *Salmonella enterica* and MRSA (17,18). As more veterinary hospitals are developing infection-control protocols, an evaluation of the effectiveness of various hand-hygiene protocols in inactivating bacteria acquired during routine hospital duties is warranted. The objectives of this pilot study were to determine the RF for bacteria on hands of students performing a routine equine physical examination associated with 3 hand hygiene protocols.

Materials and methods

Study population

Veterinary students from the Ontario Veterinary College (OVC) and Colorado State University (CSU) College of Veterinary Medicine and Biomedical Sciences were recruited as volunteer participants in this study. All students were in the 2nd through 4th y of the veterinary program and had received prior instruction on appropriate methods for performing physical examinations of horses. Students chose a horse on which to perform a physical examination out of those available on a given day. The number ranged from 2 (OVC) to 6 (CSU) horses. The horses used in this study were owned by the respective University (OVC or CSU) and were considered to be healthy other than some that had chronic lameness problems. The horses at OVC resided in pastures or paddocks during the day and were brought into tie stalls for teaching purposes. The horses at CSU resided in dry lots and were never stalled. Some of the horses had a light to moderate covering of debris on their coats but were generally free from caked or large accumulations of mud or manure.

This research was conducted with the approval of each institution's respective Human Research Committee and Animal Care and Use Committee.

Physical examination procedure

The physical examinations were performed near the horse's stabling area. Students were instructed to remove jewelry from their hands. Examination gloves were worn until the start of the physical examination to avoid any contamination of hands by the surrounding environment or the horse's lead rope or halter. The student examiner performed a standardized physical examination. This included using a pen light to examine the eyes and ears, running their hands

over all body surfaces to detect any abnormalities, examining oral mucous membranes, taking rectal body temperature, and examining the feet by picking up each foot. Examiners used a penlight, stethoscope, and digital thermometer to complete their physical examinations. This equipment was cleaned by wiping it with 70% alcohol prior to each examination. One of the authors (JLTD) observed students while they performed all physical examinations. The researchers were not masked as to the hand hygiene protocol performed by the student.

Hand sampling methods prephysical examination and postphysical examination

Samples of hand bacterial flora were collected prior to the physical examination from the left hand of each examiner. This hand was then dried with a clean paper towel. After the physical examination, a sample from the left hand was again collected and this hand was dried with a clean paper towel that was handed to them.

To collect the hand flora sample, the hand was placed into a new plastic gallon bag containing 100 mL of sterile phosphate buffered saline (PBS, pH = 7.4). The bag was grasped tightly around the examiner's wrist and the PBS was massaged over all surfaces of the examiner's hand for 30 s. The participant removed his/her hand and the bag was immediately sealed.

Hand-hygiene protocols and sampling after hand hygiene protocol

Examiners used 1 of the following hand-hygiene protocols after each physical examination was completed:

Hand-washing protocol — The examiner rubbed his or her hands together under a stream of the nearest source of running water (which was turned on by the person collecting samples), then washed for 15 s with an antiseptic soap containing 0.3% triclosan (Bacti-Stat; Ecolab Professional Division, St. Paul, Minnesota, USA) as the active ingredient. The person collecting samples then dispensed soap into the examiner's hand. Examiners were instructed to be sure to rub the soap repeatedly over all surfaces of his or her hands, including between the fingers. The examiner then dried his or her hands with a clean paper towel handed to them. The person's right hand was sampled by the post hand hygiene protocol described above.

Alcohol-gel hand sanitizer protocol — An alcohol-based gel (Purell; GoJo Industries, Alton, Ohio, USA) containing 62% ethyl alcohol as an active ingredient was applied. A dime-sized amount (approximately 1 cm in diameter) of the gel was put into the palm of the examiner's hand. The examiner then put the finger tips of his/her opposite hand into the gel, being sure that the finger tips and nails were well-coated. They wiped the gel over the entire surface of both hands. This procedure was repeated with the opposite hand. The examiner rubbed his/her hands together until they indicated that the gel had dried. The approximate time to completion of this process was 30 to 60 s. The person's right hand was sampled by the post hand hygiene protocol, described above.

Alcohol with chlorhexidine lotion — The person collecting the sample squirted 2 mL or 1 pump of the 61% ethyl alcohol (w/w) with 1% chlorhexidine gluconate moisturizer lotion (Avagard; 3M Health Care) into the examiner's palm. The examiner put his/her finger tips from the opposite hand into the lotion, being sure that the finger tips and nails were coated with the product. He or she then rubbed the product over all surfaces of both hands. This process was repeated on the other hand. The examiner rubbed both hands together until dry, approximately 30 to 60 s. The person's right hand was sampled by the post hand hygiene protocol described above.

Culture and quantification methods

The samples were processed within 1 h of collection. One hundred microliters of the original sampling fluid, as well as 100 μL from each of 2 10-fold serial dilutions of the original hand sampling PBS, were inoculated onto tryptone soya agar (TSA) plates. The plates were incubated at 35°C for 24 h. Bacterial growth was quantified via colony counts on plates containing between 30 and 300 colonies by using a hand held colony counter (Colony Counter, Model F37862-0000; Bel Art Products Scienceware, Pequanmock, New Jersey, USA). Identification and quantification for each type of bacterium of the post-hand-hygiene protocol bacterial flora were performed on the CSU samples only at the CSU Veterinary Diagnostic Bacteriology Laboratory by using standard bacteriological methods to identify bacterial types.

Data analysis

For analysis, the $\log_{10}$ bacterial counts were truncated at the lower limit of detection (3×10^4 CFU/mL). The difference in the truncated log bacterial counts was calculated between the prephysical examination counts and the postphysical examination counts (effect of physical) and between the postphysical examination counts and the posthygiene procedure counts (effect of protocol). The mean values (counts or differences) were compared by hand-hygiene protocol and study site (CSU and OVC), using analysis of variance (SAS version 9; SAS, Cary, North Carolina, USA). The distribution of the model residuals was evaluated graphically to determine if the model assumptions were appropriate. Scheffe's method was used to account for multiple comparisons when each hand-hygiene protocol was compared against the others.

Results

Quantification of bacterial load on hands

There was no difference among the prephysical bacterial loads, based on location (CSU versus OVC). The mean for the level of bacterial load increased by 0.91 $\log_{10}$ from the prephysical sample to the postphysical sample at OVC, but by only 0.36 $\log_{10}$ at CSU. There was a difference by site for the postphysical examination level of bacteria on examiners' hands, with the examiners at OVC having a higher bacterial load than those at CSU ($P = 0.003$). The mean RF between the postphysical and the posthand-hygiene protocol samples for the hand washing group was less than 0.60 $\log_{10}$ (Table 1). The mean RF between the samples was 1.29 $\log_{10}$ (CSU) and 1.44 $\log_{10}$ (OVC) for the alcohol – gel group, and 1.47 $\log_{10}$ (CSU) and 1.94 $\log_{10}$ (OVC) for the chlorhexidine-alcohol group. The RF was significantly different ($P < 0.0001$) between the hand-washing group and the other 2 treatment groups (the alcohol-gel and the

Table 1. Mean and standard error ($s_{\bar{x}}$) for the $\log_{10}$ for the bacterial colony count per hand for the prephysical, postphysical, and postprotocol for hand hygiene and reduction factor (postphysical-posthygiene protocol) and study site (CSU = Colorado State University College of Veterinary Medicine and Biomedical Sciences, OVC = Ontario Veterinary College)

	Hand hygiene protocol					
	Hand washing		Alcohol gel		Alcohol with chlorhexidine lotion	
Measurement	Mean, $s_{\bar{x}}$ CSU (n = 8)	Mean, $s_{\bar{x}}$ OVC (n = 8)	Mean, $s_{\bar{x}}$ CSU (n = 8)	Mean, $s_{\bar{x}}$ OVC (n = 8)	Mean, $s_{\bar{x}}$ CSU (n = 8)	Mean, $s_{\bar{x}}$ OVC (n = 8)
Prephysical[a]	5.44, 0.16	5.75, 0.22	5.70, 0.41	5.85, 0.16	5.37, 0.28	5.37, 0.14
Postphysical[b]	5.60, 0.07	6.79, 0.13	6.06, 0.24	6.47, 0.24	5.95, 0.19	6.42, 0.31
Postprotocol[c]	5.37, 0.15	6.20, 0.18	4.76, 0.12	5.03, 0.17	4.48, 0	4.48, 0
Reduction factor (post-physical-post-protocol for hand hygiene sample)[d]	0.22, 0.13	0.59, 0.18	1.29, 0.16	1.44, 0.10	1.47, 0.19	1.94, 0.31

[a]No difference by site or hand hygiene protocol
[b]No difference by hand hygiene protocol but difference by site $P = 0.0003$
[c]Difference by site $P = 0.003$ and hand hygiene protocol $P < 0.0001$
[d]Difference by site $P = 0.03$ and hand hygiene protocol $P < 0.0001$

chlorhexidine-alcohol lotion). There was no significant difference for the RF between the alcohol-gel groups and the chlorhexidine-alcohol lotion groups, although there was a trend ($P = 0.08$) toward the chlorhexidine-alcohol lotion being more effective.

The chlorhexidine-alcohol lotion posthand-hygiene protocol samples consistently had bacterial growth that was less than 30 colonies on the plate. During 3 of the trials with chlorhexidine-alcohol lotion, more or the same number of colonies were observed on the more sequential dilution plates than on the plate with the sample taken directly from the sampling bag.

It was beyond the scope of this study to identify every colony type on the physical examination samples and the posthand-hygiene protocol samples. However, attempts were made to identify and quantify bacteria present from the posthygiene samples obtained at CSU. The hand washing samples from the direct plating of the undiluted collection sample had colonies of coagulase negative *Staphylococcus* spp. (from 4 to 300, with a mean of 145) and *Bacillus* spp. (from 0 present to 2 with a mean of 0.9). For the alcohol-gel treatment on the direct plating, the coagulase negative *Staphylococcus* spp. ranged from 0 to 100 colonies, with a mean of 23, and *Bacillus* spp. ranged from 0 to 38 colonies, with a mean of 15. There was 1 sample from the posthand-hygiene alcohol-gel group that had *Corynebacterium* spp. at a level too numerous to count on the plate with the undiluted sample. For the alcohol-chlorhexidine lotion group, the number of *Staphylococcus* spp. colonies ranged from 0 to 8, with a mean of 2, and the *Bacillus* spp. colonies ranged from 0 to 12, with a mean of 4. In summary, the posthand washing samples had a larger proportion of the colonies that were coagulase negative *Staphylococcus* spp., while the chlorhexidine-alcohol lotion group had a larger proportion of the colonies that were *Bacillus* spp. The alcohol-gel group had about equal proportions of coagulase negative *Staphylococcus* spp. and *Bacillus* spp.

Discussion

Our goal in performing this study was to compare the effectiveness of hand-hygiene protocols that seemed practically relevant to typical veterinary clinical settings. While other methods or products could have been compared, we chose to emphasize protocols that had a high likelihood for use in typical practice settings.

Based on results of this study, hygiene protocols implementing the alcohol-based gel or the chlorhexidine-alcohol lotion were apparently as or more effective when compared with hand washing for 15 s with an antibactieral soap. Several factors need to be considered when interpreting findings from this study.

The alcohol-based gel product had a concentration of active ingredient that is typical of most of the alcohol-based gels that are commercially available in the USA (60% to 68% alcohol). There is now at least 1 product on the market that contains a higher concentration of alcohol in a gel formulation. There would be merit in evaluating the efficacy of this product compared with those that are now in common use and in evaluating alcohol liquid products. Some have proposed that these liquid products are more effective than the gel products (8). In some published trials of hand washing effectiveness, the length of time evaluated for washing has been from 30 to 60 s (8). While a longer period might be optimal and could have reduced the bacterial loads further, a hand washing duration of 15 s was chosen, as this was considered to be more typical of actual hand washing practices used in veterinary clinical settings. In fact, the authors' experiences suggest that the hand washing times actually used in veterinary settings are frequently less than 15 s. It is also possible that resistance to the antibacterial agent in the soap may have affected results, as resistance to triclosan has been demonstrated (8).

The alcohol-based products performed relatively well in reducing overall bacterial load in this experiment, despite being clearly labeled as "not for use on grossly soiled hands." This is an important finding for ambulatory practitioners, who may not always have access to hand washing facilities.

The prephysical examination hand bacterial loads for persons in this study were similar to those previously reported for human health care workers (19). The higher bacterial counts on the hands postphysical examination of

OVC students compared with CSU students could have been due to the horses at OVC having a higher bacterial load on their bodies, the students at OVC touching the horses more frequently or in more places, the hands of students at OVC as a group being larger and thus having more surface area to acquire bacteria, or some other factor. As part of the Human Research Council protocol, we were unable to identify the students who participated and correlate this information with their test result, so impact of gender on hand size and thus bacterial load could not be evaluated.

On several occasions, there was no bacterial growth from the hand samples, indicating that if bacteria were present, they were below the level of detection. If the endpoint in the chlorhexidine-alcohol lotion group had not been fixed, a difference between the alcohol-gel group and the chlorhexidine-alcohol lotion group might have been apparent, but we were conservative in our evaluation of the bacterial counts. In these experiments, no treatment was applied to inactivate any one of the hygiene products when it was plated onto the TSA, since this is the solution as it would have remained on the examiners hands posttreatment. The fact that, 3 samples of the chlorhexidine-alcohol lotion protocol had an approximately equal number of bacterial colonies on subsequent 10-fold dilution plates, as on the direct plate, was due possibly to dilution of the hand-hygiene product in the sample.

In this pilot study, the types of bacteria on hands after hand hygiene were not fully evaluated, nor was any testing done to detect the efficacy of these hand-hygiene protocols in inactivating viruses. In a subset of the samples, the types of bacteria left on hands after hand washing appeared to be those that could represent resident flora (coagulase-negative *Staphylococcus* spp). The alcohol gel and chlorhexidine-alcohol lotion eliminated a larger number of *Staphylococcus* spp. but allowed *Bacillus* spp. to remain.

Further work is needed to explore the types of bacteria left on examiners' hands, particularly in terms of the clinical relevance of these organisms. This would entail subculture and definitive identification of all colony types on all posthygiene samples. Based on the overall reduction in colony numbers and types of bacteria present after hand-hygiene protocols at CSU, it is likely that transient enteric bacteria would have been removed with any of the treatments, but it is difficult to be sure, as very few enteric types of bacteria were identified with the test methods used. In future studies, enrichment methods could be utilized to detect and determine the type of more fastidious organisms, such as *Salmonella* spp.

We believe that students and the examinations they performed were broadly representative of the veterinary student population and the types of examinations they performed were typical of a thorough examination usually performed during the initial hospitalization of an equine patient.

A $\log_{10}$ RF of 1.0 equates to a 90% reduction, a 2.0 $\log_{10}$ equates to a 99% reduction in, and 3.0 $\log_{10}$ to a 99.9% reduction in bacterial colony numbers (8). The level of reduction necessary to prevent cross contamination is not known for hospitalized human patients and certainly is not known for veterinary patients. Thus, we cannot be certain if the level of reduction in bacterial load observed in these experiments would be adequate to prevent nosocomial spread of bacteria between veterinary patients. Most of the products approved for the health-care setting reduce the bacterial load by approximately 99%. Some of the remaining bacteria could pose a risk to patients and, certainly, in known high-risk situations, the wearing of disposable gloves when examining patients, followed by hand washing or sanitation, would be indicated.

Equally important to the efficacy of the product is the need to emphasize compliance by the health-care workers. It has been stated by several sources that the compliance of health-care workers with hand-hygiene protocols was better with gels than with other forms of hand hygiene (8,9,13). From a clinical perspective, if hand gels or lotion reduces overall bacterial counts equal to or better than hand washing with an antiseptic soap, as shown in this study, perhaps they should be considered for use in situations where hand washing cannot be practised, where hand hygiene needs to be optimal (in high-risk situations), or in conjunction with hand washing.

There are multiple factors that make the need for and application of hand hygiene different in the equine veterinary practice area than in human medicine; hand alcohol-gel products are not appropriate for use when hands are visibly dirty or soiled (8). Many equine veterinary practitioners work in conditions where their hands will be visibly soiled, yet they are not in a position to wash their hands prior to seeing their next patient, handling equipment or drug containers within their practice vehicle, or both. The alcohol-based gels or chlorhexidine-alcohol products must still stand the test of time in the equine hospital and equine ambulatory practice setting, so that they can be fully assessed as to how effective they will be in the real-world situation for reducing the spread of disease agents among patients and in protecting health-care workers from exposure to zoonotic agents that could be spread by hand contamination. In this study, they performed as well if not better than hand washing for reduction in bacterial load on the hands of people performing a routine physical examination. Where optimal hand hygiene would be indicated, such as in a veterinary clinic, alcohol-gel or chlorhexidine-alcohol lotion products could be implemented to supplement routine hand washing or used in place of hand washing when hands are not visibly soiled.

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Book Review
Compte rendu de livre

Color Atlas of Diseases and Disorders of Cattle, 2nd ed.

Blowey RW, Weaver AD. MOSBY, Elsevier Science Limited, Edinburgh, Scotland, 2003, ISBN O-7234-3205-8.

The authors of this text are to be congratulated on their revised edition which should greatly improve the utility of this text for all readers, both students and experienced diagnosticians, wherever in the world they may be located. In this 2nd edition, the inclusion of brief clinical descriptions to provide a context within which to view and interpret the many excellent photographs is a welcomed addition. Further, the authors have shown considerable discipline and restraint by keeping these descriptions concise, which is in keeping with the stated purpose of the text to provide an atlas, not a textbook, on bovine diseases.

Although in general the photographs clearly depict the problem or lesion being discussed, there are a few instances where the use of arrows to specifically outline the lesion would have been helpful for the inexperienced clinician, student veterinarian, and, in particular, the agricultural student and livestock producer namely p23 #76, p30 #103, p48 #161, p152 #523, and p158 #542. Also, the authors make use of many acronyms, such as CF, AGID, PPH, and P-O, without first writing the words or terms out in full or including them in the index. Preclinical veterinary students and, in particular, agricultural students and livestock producers may find this a frustrating problem not easily solved, especially if she or he does not have ready access to other large animal veterinary medicine texts. Possible solutions that the authors might consider are to avoid using acronyms in the text, the inclusion of all acronyms in the index, or a list or glossary of all acronyms included in the text.

In conclusion, the targeted users should find this 2nd edition a very useful addition to their library.

Reviewed by ***Danny Butler, DVM, MSc, PhD,*** *Department of Clinical Studies (Retired), Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.*